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Biomineralized Materials for Sustainable and Durable Construction

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Abstract

Portland cement concrete, the most used manufactured material in the world, is a significant contributor to anthropogenic carbon dioxide (CO_2) emissions. While strategies such as point-source CO_2 capture, renewable fuels, alternative cements, and supplementary cementitious materials can yield substantial reductions in cement-related CO_2 emissions, emerging biocement technologies based on the mechanisms of microbial biomineralization have the potential to radically transform the industry. In this work, we present a review and meta-analysis of the field of biomineralized building materials and their potential to improve the sustainability and durability of civil infrastructure. First, we review the mechanisms of microbial biomineralization, which underpin our discussion of current and emerging biomineralized material technologies and their applications within the construction industry. We conclude by highlighting the technical, economic, and environmental challenges that must be addressed before new, innovative biomineralized material technologies can scale beyond the laboratory.

1. INTRODUCTION

1.1. Climate Change and the Built Environment

The construction industry accounts for 39% of global carbon dioxide (CO_2) emissions (1), of which 11% can be attributed to embodied CO_2 equivalent emissions, which encompass all construction materials–related CO_2 emissions from sourcing, manufacturing, use, and disposal. Due to the anticipated increase in construction over the next three decades and the CO_2 emissions intensity of the most common building and construction materials (i.e., concrete, steel, glass, and aluminum), it is estimated that embodied CO_2 emissions will account for 57% of total new construction emissions by 2050 (1, 2). The production of portland cement concrete accounts for 8% of global CO_2 emissions (3, 4); the production of portland cement alone accounts for 7%.

Between now and 2050, the International Energy Agency (IEA) predicts that the global demand for concrete will increase by 12–23%, which will lead to increased demand for portland cement, as well as to increased CO_2 emissions related to global cement production (**Figure 1**) (5–8). China is predicted to remain the largest cement producer through 2050. However, China's share of production is expected to decrease beyond 2050 as other developing regions, particularly South Asia and Africa, increase their cement production. Although process-related CO_2 emissions are expected to increase over time with increases in portland cement demand, the 2°C scenario outlined in the IEA's cement technology roadmap (5) anticipates reductions in process-related emissions between now and 2050 through innovations that are described in Section 1.3.

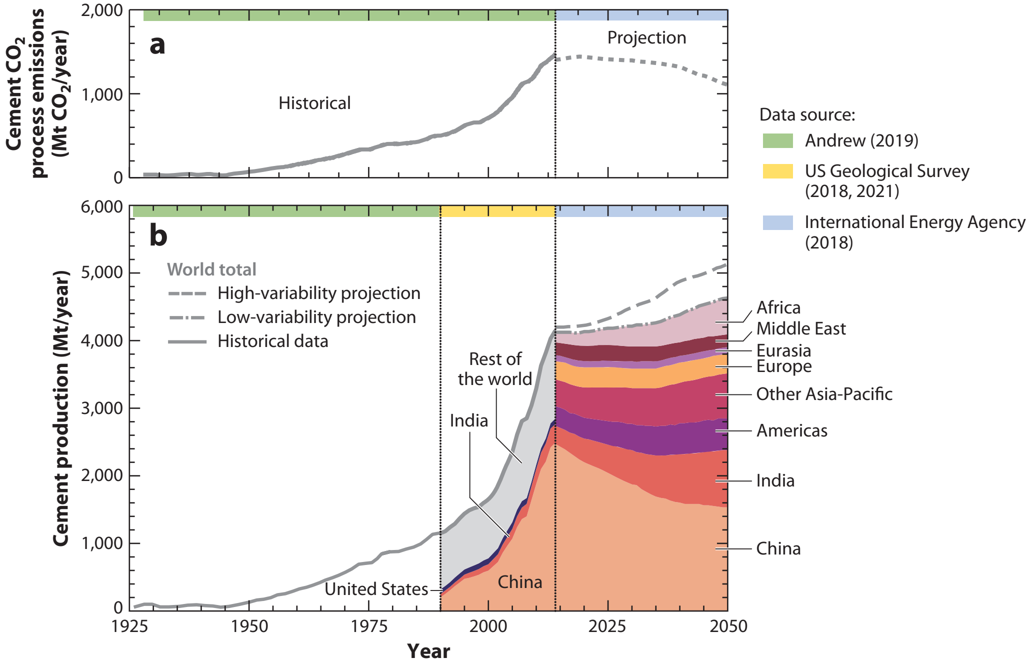

Figure 1

(*a*) Process-related CO_2 emissions associated with (*b*) portland cement production from 1927 to 2050. Historic values (*solid lines*) are taken from Andrew et al. (6) (CO_2 emissions 1928–2014 and cement production 1926–1990, *green bars*) and the US Geological Survey (7, 8) (cement production 1990–2014, *yellow bar*). Projected values are taken from the International Energy Agency's cement technology roadmap (5) (CO_2 emissions and cement production 2014–2050, *blue bars*).

1.2. Production of Portland Cement

Conventional portland cement is a powdered hydraulic cement composed of ground clinker (~95%) and a small amount of gypsum (~5%). Clinker is produced by sintering natural, quarried, or mined materials composed of calcium carbonate ($CaCO_3$), alumina (Al_2O_3), silica (SiO_2), and iron oxide (Fe_2O_3) at high temperatures in cement kilns. The main reactions that take place in cement kilns are calcination (i.e., the conversion of $CaCO_3$ to CaO and CO_2) and the reaction of CaO with Al_2O_3, SiO_2, and Fe_2O_3 to form imperfect solid-solution calcium silicate, aluminate, and ferrite phases. The main clinker phases that form include alite (~Ca_3SiO_5) (48–68%), belite (~Ca_2SiO_4) (6–27%), calcium aluminate (~$Ca_3Al_2O_6$) (0–12%), and calcium aluminoferrite [$Ca_2(Al,Fe)_2O_5$] (4–13%) (9). When reacted with water, alite and belite form calcium silicate hydrate, the reaction product that is responsible for the high compressive strength of portland cement concrete. Gypsum ($CaSO_4{\cdot}2H_2O$) (~5%) is added to ground clinker to regulate the setting time of portland cement. Gypsum inhibits flash setting of the calcium aluminate phases so that the concrete can remain workable and moldable for a period of hours instead of minutes to enable transport and placement.

Concrete is composed of portland cement, water, and aggregate, including fine aggregate (i.e., sand) and coarse aggregate (i.e., rocks). Portland cement and water chemically react to form a binder that holds the fine and coarse aggregates together. The advantages of portland cement concrete—its moldability, pumpability, strength, durability, cost, and global availability—have made it the most widely used and the most environmentally impactful construction material on Earth.

The CO_2 emissions directly related to the cement production process can be classified into two categories: fuel-related emissions and calcination-related emissions (5). Fuel-related emissions account for ~30–40% of direct CO_2 emissions associated with portland cement production. Cement kilns must be heated to 1,450°C to facilitate clinker reactions; currently, these temperatures are achieved almost entirely by the burning of fossil fuels. CO_2 is also released during cement production when limestone ($CaCO_3$) is calcined to CaO. Calcination-related emissions account for ~60–70% of direct CO_2 emissions. Together, these process-related CO_2 emissions currently account for almost all of the emissions released during the production of portland cement.

1.3. Alternatives to Portland Cement Concrete

Over the past few decades, a concerted, global research effort has yielded innovations that aim to reduce the environmental impact of portland cement concrete (3–5). Innovations include (*a*) emissions reductions through point-source CO_2 capture and storage at cement plants, (*b*) reduced clinker content in concrete through the use of supplementary cementitious materials and alternative cements [e.g., limestone-calcined clay cements, alkali-activated cements, geopolymer cements (**Figure 2**)], (*c*) injection or exposure of concrete to CO_2 during curing to accelerate carbonation and resorption, (*d*) use of alternative fuels, and (*e*) increased energy efficiency of the cement production process (5). Other strategies that reduce portland cement consumption include (*a*) structural design innovations that help avoid overdesign, (*b*) use of ultrahigh-performance concretes that enable smaller volumes of structural elements (e.g., beams, columns), and (*c*) the use of CO_2-storing mineral aggregates in concrete production.

Although each of these emissions-reduction strategies will play a key role in reducing CO_2 emissions, more disruptive approaches that have the potential to radically transform the cement and concrete industry are also being explored. The most transformative of such approaches leverages biological processes and, in particular, microbial biomineralization to produce more durable portland cement concrete (i.e., self-healing concrete). Microbial biomineralization is also being

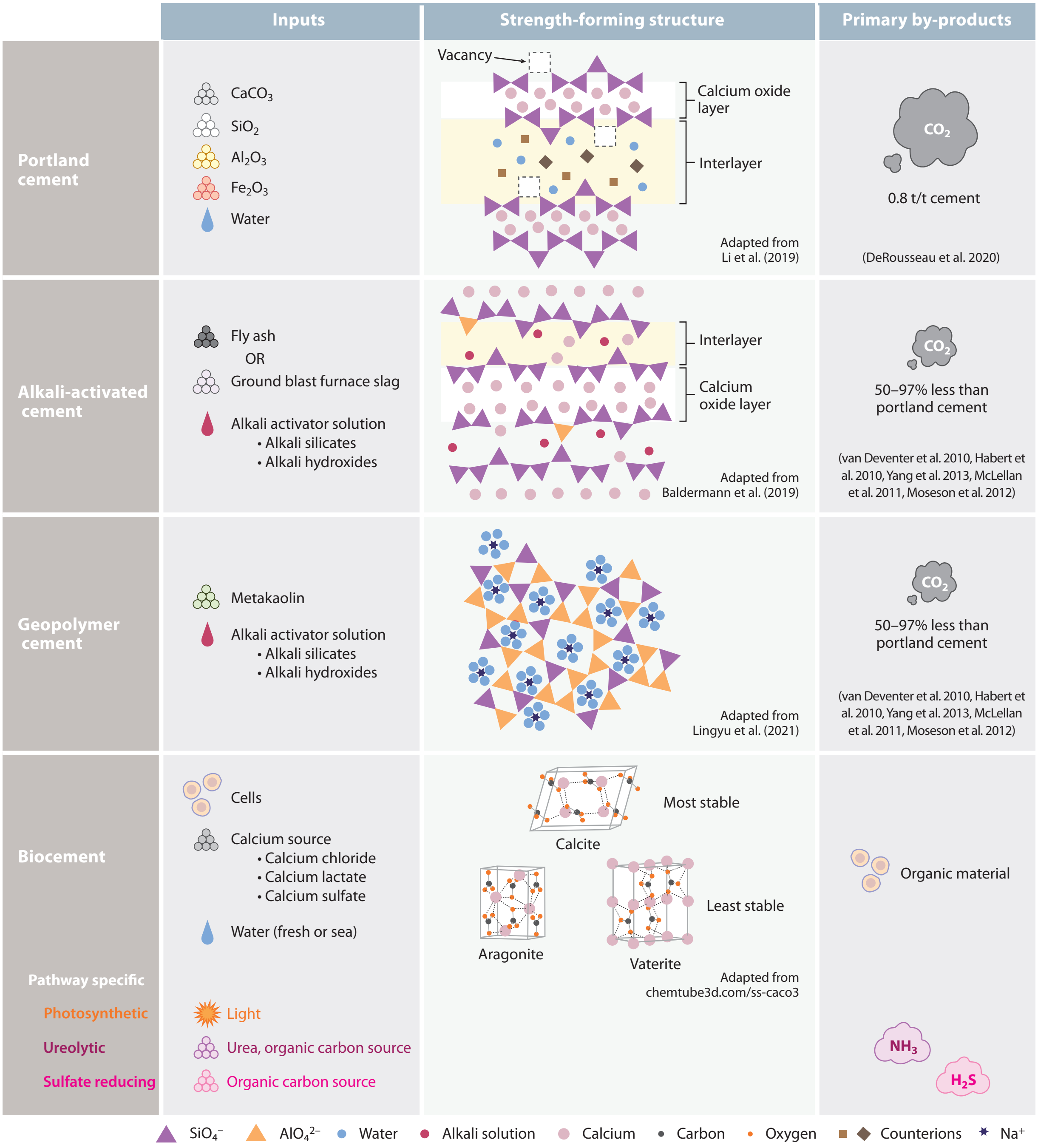

Figure 2

Schematics of inputs, strength-forming structures, and primary by-products [DeRousseau et al. (14), van Deventer et al. (15), Habert et al. (16), Yang et al. (17), McLellan et al. (18), and Moseson et al. (19)] for different types of cementitious materials. Portland cement strength-forming structure adapted with permission from Li et al. (10); copyright 2019, American Chemical Society. Alkali-activated cement strength-forming structure adapted from Baldermann et al. (11) (CC BY 4.0). Geopolymer strength-forming structure adapted from Lingyu et al. (12) (CC BY 4.0). Biocement strength-forming structure adapted from ChemTube3D (13).

exploited to preserve existing structures, stabilize soils, and produce sustainable and durable alternatives to portland cement concrete.

Biomineralization-inspired approaches leverage the metabolic activity of living microorganisms or their active enzymes. The metabolic processes of biomineralizing organisms result in the formation of biominerals. $CaCO_3$ is the most common biomineral precipitated by biomineralizing organisms. In self-healing portland cement concrete, $CaCO_3$ can act as a cementing material to help seal cracks, increase strength, and decrease water absorption. Biominerals can also act alone as a biocement (**Figure 2**) that binds aggregate particles together to create load-bearing concrete-like materials, supplanting the need for portland cement altogether.

While construction applications of microbial biomineralization have been explored over the past few decades (20–24), a principal challenge lies in ensuring the long-term viability and metabolic activity of the microorganisms over time. The pore solution chemistry of portland cement concrete is particularly extreme for biomineralizing microorganisms that are directly added to the cementitious matrix to impart self-healing capabilities. High pH values, high temperature during portland cement hydration, and lack of oxygen and nutrients within cementitious materials reduce microorganism viability if they are directly added to concrete, thereby limiting their biomineralization functionality (20, 25–27). Viability dictates which microorganisms are selected, how they are incorporated into construction materials, and what strength or long-term durability improvements are enabled by their addition.

In this article, we critically review the field of biomineralized construction, including both traditional (e.g., self-healing concrete) and emergent biomineralized material technologies and their applications, limitations, and challenges. First, we review the mechanisms of microbial biomineralization, identify the principal microorganisms preferred by researchers, and discuss their advantages and disadvantages. Next, we review construction applications of biomineralized materials, including historic preservation, soil stabilization, and self-healing concrete. A meta-analysis is presented to show variability in compressive strength and water absorption when microorganisms are added directly to building materials for applications such as self-healing concrete, biomineralized concrete-like alternatives, and other biomineralized building materials. Emerging applications and techniques for creating biomineralized building materials are highlighted, as are the main limitations and challenges that the field must overcome before biomineralized materials can scale beyond the laboratory and find application within the commercial sector.

2. FUNDAMENTALS OF BIOMINERALIZATION

2.1. Biologically Induced and Biologically Controlled Mineralization

Biomineralization is the process by which minerals are precipitated from the environment through the natural processes of living microorganisms. Biomineralization is either a passive or an active process, depending on the organism and its surrounding environment. Passive biomineralization is often termed biologically induced mineralization, whereas active biomineralization is termed biologically controlled mineralization (**Figure 3**) (20, 21, 23, 28).

Biologically induced mineralization occurs when the normal biological processes of living organisms (e.g., bacteria) cause changes to the surrounding environment (**Figure 3*a***). These changes, such as changes in pH and the release of metabolic by-products, interact with compounds in the surrounding environment to induce precipitation of biominerals in an uncontrolled manner. This precipitation can occur on a cell's surface as well as on nearby biological materials or nonliving particles.

During biologically controlled mineralization, living organisms actively direct the precipitation of biominerals (**Figure 3*b***). Biomineral composition, phase, morphology, nucleation, and growth

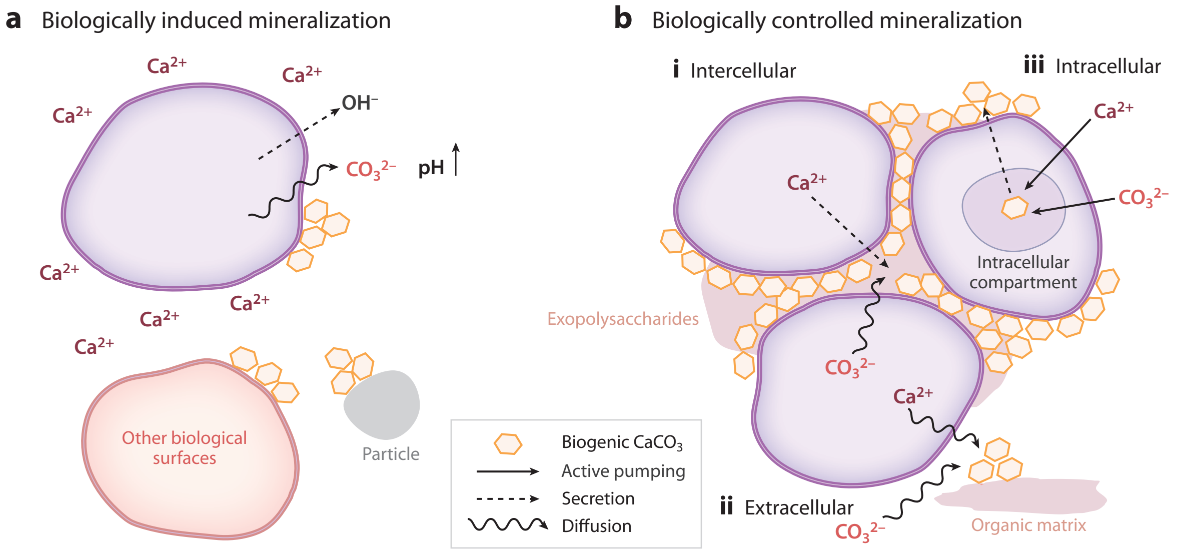

Figure 3

Schematics of (*a*) biologically induced and (*b*) biologically controlled mineralization, showing (*i*) intercellular, (*ii*) extracellular, and (*iii*) intracellular processes. Biologically induced mineralization occurs when environmental changes (such as changes in pH and the release of metabolic by-products caused by normal biological processes of living organisms) lead to the uncontrolled precipitation of biominerals. During biologically controlled mineralization, living organisms actively direct the precipitation of biominerals. Biologically controlled mineralization can occur on external organic matrices (*red materials*) after passive diffusion of ions from inside a biomineralizing cell (*purple cells*). (*b*, *ii*) Biologically controlled mineralization can also occur on biomineralizing cell surfaces after the controlled secretion of ions from within a biomineralizing cell (*b*, *i*) or within specialized compartments within biomineralizing cells after the controlled pumping of ions into the cell from the external environment (*b*, *iii*). Following intracellular mineralization, minerals are secreted to the outside surface of the biomineralizing cell. Figure adapted from Castro-Alonso et al. (28) (CC BY 4.0).

are all directly controlled during biologically controlled mineralization (28). This controlled mineralization can occur extracellularly (e.g., in mollusks, bryozoans, bones, and teeth), intercellularly (e.g., in calcareous algae), or intracellularly (e.g., in haptophyte algae) (29). Organic macromolecules, such as proteins and exopolysaccharides, often assist in the controlled formation of biominerals. During extracellular biologically controlled mineralization, biominerals are precipitated in external organic matrices after the passive diffusion of ions from inside cells (**Figure 3*b*,*ii***). Intercellular biologically controlled mineralization involves the controlled secretion of ions from inside a cell; cell surfaces then act as substrates for biomineralization (**Figure 3*b*,*i***). Intracellular biomineralization is the most tightly regulated of these processes. Specific ions are actively pumped into cells from their external environment. Inside the cell, specialized internal compartments actively combine transported ions into specific mineral phases; these minerals are then secreted to the outside surface of the cell (**Figure 3*b*,*iii***).

A variety of minerals are precipitated through passive or active biomineralization in the natural environment. These precipitated minerals include carbonates, phosphates, silicates, and metal-containing complexes such as iron-based minerals (30–33). Construction materials research has focused on biomineralization of metal-containing complexes specifically for remediation of contaminated sites (34, 35). For sustainable construction materials, and specifically for sustainable cement and concrete research, polymorphs of $CaCO_3$ are the most widely researched biominerals

(22, 24, 36). Researchers have also explored calcium phosphates [$Ca_3(PO_4)_2$] for biocement applications, but $CaCO_3$ remains the predominant focus of biocement research (30, 31).

During biomineralization processes, $CaCO_3$ can precipitate in various polymorphs. These polymorphs include trigonal calcite, hexagonal vaterite, orthorhombic aragonite, and amorphous $CaCO_3$ phases (37). Which phase preferentially precipitates is a function of the microorganism selected, the mineralization pathway, and the environmental conditions. Amorphous $CaCO_3$ and vaterite are less stable than calcite and aragonite and are often precursor and metastable phases, respectively, during the biomineralization process. Recently, researchers have begun exploring the controlled morphological precipitation of $CaCO_3$ polymorphs through environmental control and genetic engineering (37). In the field of construction biotechnology, biomineralization of $CaCO_3$ is often referred to as microbially induced calcite precipitation (MICP). MICP is often used to refer to biologically induced mineralization as opposed to biologically controlled mineralization.

2.2. $CaCO_3$ Biomineralization Pathways

$CaCO_3$ biomineralization occurs through numerous pathways, as reviewed by Mondal & Ghosh (38). Urea hydrolysis is the most studied and best understood of these pathways but requires significant nutrient addition. Photosynthesis requires low nutrient addition and sequesters CO_2 but also requires continuous access to light and CO_2. Sulfate reduction is significantly less studied in construction materials research, as are other biomineralization pathways, such as nitrate reduction and organic compound conversion.

2.2.1. Urea hydrolysis. $CaCO_3$ biomineralization through urea hydrolysis is the most widely applied approach in construction materials research (21–23, 25, 28, 38). During microbial urea hydrolysis, microorganisms produce the urease enzyme. This enzyme hydrolyzes urea $CO(NH_2)_2$ (Reaction 1), which ultimately leads to the production of carbonate ($CO_3{}^{2-}$) and ammonium ($NH_4{}^+$) ions. If dissolved Ca^{2+} is present, $CO_3{}^{2-}$ ions then react with Ca^{2+} ions to produce $CaCO_3$ (Reaction 5).

$$CO(NH_2)_2 + 2H_2O \xrightarrow{\text{Urease enzyme}} H_2CO_3 + 2NH_3, \qquad 1.$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+, \qquad 2.$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^-, \qquad 3.$$

$$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O, \qquad 4.$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3. \qquad 5.$$

Typically, ureolytic biomineralizing microorganisms have a negative cellular surface charge, which attracts positive Ca^{2+} ions in solution and leads to the precipitation of $CaCO_3$ at the cellular surface. This mechanism has been widely exploited in materials science research due to its efficient and fast production of $CaCO_3$ at the laboratory scale (22). Ureolytic microorganisms are also highly resilient; they are often alkaliphilic, spore-forming, gram-positive microorganisms isolated from soil environments. Due to this high resilience, ureolytic microorganisms better withstand some of the extreme environmental pressures within cement and concrete materials. Their ability to form spores may allow them to survive over longer periods of time within materials that contain harsh physical and chemical environments (e.g., the pore solution of portland cement concrete) relative to non-spore-forming microorganisms. Thus, ureolytic microorganisms have been heavily utilized for self-healing portland cement concrete applications.

Alternatively, an emerging strategy is to use the urease enzyme alone in cell-free enzymatic biomineralization applications (39). The principal advantage of using ureolytic enzymes is that enzymes are more resistant than living cells to environmental parameters (e.g., soil type in soil stabilization applications), so urea hydrolysis and biomineralization continue longer and may reach higher biomineralization efficiencies than are achieved with living microorganisms. The environmental impact of the ureolytic biomineralization pathway may also be reduced through the use of the urease enzyme alone because ammonia is not released during enzymatic biomineralization. The urease enzyme also degrades naturally over time, eliminating the concern of releasing non-native microorganisms and their associated metabolic by-products to the environment. However, the cost of enzyme production is currently a significant hindrance to the use of enzymatic biomineralization in construction applications (39).

2.2.2. Photosynthesis. Biomineralization of $CaCO_3$ through photosynthesis is much less studied and rarely applied in construction materials research. However, interest in the use of photosynthetic microorganisms for construction biomineralization applications is increasing (38, 40, 41). During photosynthetic respiration, assimilated CO_2 in the form of glucose is rereleased as a by-product of the production of energy (ATP). This rereleased CO_2 reacts with water to produce carbonic acid (H_2CO_3) (Reaction 6). H_2CO_3 then dissociates in solution to produce bicarbonate ions (HCO_3^-) (Reaction 7), which subsequently react with Ca^{2+} ions in solution to precipitate $CaCO_3$ (Reaction 8) (38).

$$CO_2 + H_2O \xrightarrow{\text{CA enzyme}} H_2CO_3, \quad 6.$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+, \quad 7.$$

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+. \quad 8.$$

As shown in Reaction 6, the enzyme carbonic anhydrase (CA) acts as a catalyst for converting CO_2 and water into HCO_3^- (21, 42). Although further research is needed to fully understand this process, it is generally understood that the production of HCO_3^- in the presence of Ca^{2+} at an appropriate pH leads to the precipitation of $CaCO_3$. While CA plays a main role in biomineralization through photosynthesis (28), it is also thought to influence biomineralization as both an enzymatic catalyst and a structural protein in a range of other pathways (42):

$$CA - H_2O \leftrightarrow CA - OH^- + H^+, \quad 9.$$

$$CA - OH^- + CO_2 + H_2O \leftrightarrow CA - H_2 + HCO_3^-, \quad 10.$$

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+. \quad 11.$$

Photosynthetic $CaCO_3$ biomineralization has been gaining attention in recent years due to the potential to sequester CO_2 during cell growth and the biomineralization process (43). As cells grow, assimilated carbon is used to produce energy and biomass. Through the reaction of rereleased CO_2 and water, additional CO_2 is then permanently fixed in biomineral form. Thus, photosynthesis presents a potentially more sustainable pathway to produce biomineralized building materials than does urea hydrolysis.

2.2.3. Sulfate reduction. Biomineralization through sulfate reduction is significantly less studied in construction materials research (21, 22, 38, 44). It can occur through two pathways. In the first mechanism, calcium sulfate ($CaSO_4$) abiotically dissociates (Reaction 12). Sulfur-reducing microorganisms then reduce sulfate to hydrogen sulfide (H_2S) (Reaction 13). During this process, HCO_3^- is released and reacts with Ca^{2+} in solution to precipitate $CaCO_3$ (Reaction 14). In the second mechanism, sulfate-reducing bacteria produce calcium sulfide (CaS) during the reduction of $CaSO_4$ (Reaction 15). CaS then reacts with water to produce calcium hydroxide [$Ca(OH)_2$] (Reaction 16), which reacts with H_2CO_3 to precipitate $CaCO_3$ (Reaction 18). Sulfate-reducing bacteria have been isolated from highly alkaline regions, which may increase their usefulness in the high-pH environment of portland cement concrete (44).

$$CaSO_4 \cdot 2H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 2H_2O, \quad 12.$$

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- + CO_2 + H_2O, \quad 13.$$

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+. \quad 14.$$

$$CaSO_4 + 2CH_2O \rightarrow CaS + 2CO_2 + 2H_2O, \quad 15.$$

$$CaS + 2H_2O \rightarrow Ca(OH)_2 + H_2S, \quad 16.$$

$$CO_2 + H_2O \rightarrow H_2CO_3, \quad 17.$$

$$Ca(OH)_2 + H_2CO_3 \rightarrow CaCO_3 + 2H_2O. \quad 18.$$

2.2.4. Advantages and disadvantages of principal biomineralization pathways. Urea hydrolysis, photosynthesis, and sulfate reduction pathways to $CaCO_3$ biomineralization have unique advantages and disadvantages (45). Urea hydrolysis is well studied. However, ureolytic microorganisms are heterotrophic and require both organic carbon (e.g., yeast extract) and urea to mineralize $CaCO_3$. This nutrient addition may affect material properties when incorporated into building materials and may prove to be a cost barrier in production scale-up. Urea hydrolysis also produces ammonia as a by-product; ammonia is considered an environmental pollutant and may lead to less efficient biomineralization (25, 31, 46).

Conversely, photosynthetic biomineralizing microorganisms are autotrophic organisms, which naturally fix CO_2 during normal metabolic and biomineralization processes. Photosynthetic organisms often require few exogenous nutrient additions. However, photosynthetic microorganisms are aerobic and require light to grow and to produce biominerals, which may limit their application (47).

One major drawback of $CaCO_3$ biomineralization through sulfate reduction is that it produces toxic H_2S gas. Sulfate-reducing microorganisms are also heterotrophic and require organic carbon nutrient addition but do not require additional resources (e.g., urea) apart from calcium sulfate (both the calcium and the sulfate source). Sulfate-reducing organisms are typically gram negative and, therefore, may be less resilient than many ureolytic microorganisms. While ureolytic microorganisms are aerobic and require continuous access to oxygen, sulfate-reducing organisms are anaerobic—a characteristic that may increase their applicability in some construction applications.

2.2.5. Other $CaCO_3$ biomineralization pathways. Additional $CaCO_3$ biomineralization pathways have been exploited in recent years. These pathways include denitrification and organic compound conversion (22, 38). During denitrification, anaerobic microorganisms reduce nitrate to nitrogen gas (Reaction 19). This reduction, when carried out in the presence of CO_2 and Ca^{2+} ions, induces the precipitation of $CaCO_3$ (Reaction 20).

$$4NO_3^- + 5CH_2O \xrightarrow{\text{Denitrification}} 2N_2 + 5CO_2 + 3H_2O + 4OH^-, \quad 19.$$

$$Ca^{2+} + CO_2 + 2OH^- \rightarrow CaCO_3 + H_2O. \quad 20.$$

Denitrifying microorganisms have been used in recent research to increase strength and inhibit corrosion through biomineralization processes in portland cement concrete (48). However, denitrifying microorganisms are much less utilized in biomineralized construction materials research than microorganisms relying on other pathways, such as urea hydrolysis. A lack of applications is mainly due to a lack of background biological understanding of denitrifying microorganisms, specifically concerning which organisms are most efficient at denitrification and how these organisms directly contribute to the biomineralization process (49). During organic compound conversion, microorganisms can directly convert organic compounds, such as calcium lactate, to $CaCO_3$ (38):

$$CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O. \quad 21.$$

Organic compound–converting microorganisms, such as *Bacillus cohnii*, have been utilized in a variety of construction materials research studies due to their ability to utilize calcium-containing organic compounds, such as calcium lactate, as both heterotrophic carbon and calcium sources for biomineralization (38, 46, 50, 51).

2.3. Application of $CaCO_3$ Mineralization in Construction Materials

To utilize biomineralization to produce sustainable and durable construction materials, two application approaches, bioaugmentation and biostimulation, are possible. Bioaugmentation involves the addition of microbial cultures to a construction material during the manufacturing process (24, 52). Due to the higher level of control and selection of specific microorganisms, the majority of construction biotechnology research relies on bioaugmentation. The second technique, biostimulation, involves the addition of nutrients to selectively stimulate indigenous microorganisms (53). Although there is less control over which organisms are targeted during biostimulation, the addition of specific nutrients can target the stimulation of certain microbial metabolism and biomineralization pathways. While less commonly exploited than bioaugmentation, biostimulation offers advantages at the industrial scale, such as lower cost, enhanced ease of application, and lower environmental safety concerns. Examples of each are presented in Section 3.

While a wide variety of biomineralizing microorganisms have been used in construction materials science research, species associated with the genus *Bacillus* are by far the most utilized (54, 55). *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*), the most common biomineralizing microorganism, is a nonpathogenic, ureolytic bacterium that has become the model organism for construction biomineralization research due to its well understood biomineralization mechanism, high urease activity, resilience (due to its ability to form spores), and high yield of $CaCO_3$. As reviewed by Chuo et al. (54), other ureolytic strains used in construction materials science research include, but are not limited to, *Bacillus sphaericus*, *Bacillus megaterium*, and *Bacillus cereus*.

Common nonureolytic biomineralizing microorganisms used for construction materials research include *Bacillus mucilaginosus*, which produces CA; various strains of photosynthetic cyanobacteria, which produce CA; and *B. cohnii*, which is an organic compound–converting microorganism. Denitrifying bacteria, such as *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens*, have also been explored, as has *Desulfovibrio bizertensis*, a sulfate-reducing bacterium. *Bacillus subtilis*, which can produce minerals through both ureolytic and nonureolytic biomineralization pathways, has also been explored for construction materials research.

Strains utilized in construction biomineralization research are most often model organisms purchased through companies or biobanks that maintain and distribute well studied and relatively well understood organisms. In recent years, however, researchers have begun to explore new strains of microorganisms isolated from natural sources (54). Microbial isolation offers various benefits over the use of biobank organisms for construction materials science research. A main benefit concerns microbial adaptation to environments specific to construction. Microorganisms isolated from locations or materials local to a construction site will already be well adapted to local environmental conditions (e.g., temperature, humidity, pH). Researchers have most often isolated new biomineralizing microorganisms from soils and sediments that contain high quantities of naturally occurring, ureolytic microorganisms (56–64). Correspondingly, *Bacillus* species and, specifically, *S. pasteurii* have often been isolated from soils and used in construction materials research (47, 60, 65–68). Microorganisms isolated from other natural environments with extreme properties (e.g., high temperature, alkalinity, and salinity) can exhibit additional adaptation and perhaps improved viability in the elevated pH and temperature environments of cement and concrete. Microorganisms have been isolated from salt flats, hot springs, and acid mire water and used in construction biomineralization research (44, 69, 70). Researchers have also directly isolated microorganisms from portland cement and portland cement concrete (47, 65, 66, 71, 72). Useful microorganisms for biomineralization research have also been isolated from human urine (73) and steel slag (74).

Apart from the benefits of utilizing locally isolated microorganisms, the process of strain isolation, understanding the actual biomineralizing mechanisms of isolated strains, and optimizing the growth parameters are important considerations in the choice to use an isolated versus a biobank-procured microorganism. Studies that seek to use ureolytic microorganisms without identifying the specific microbial strain through 16S rRNA sequencing (or similar) (62–64), for example, are at a particular disadvantage in terms of fundamental mechanistic understanding, control, and process optimization.

3. BIOMINERALIZED MATERIALS: STATE-OF-THE-ART CONSTRUCTION APPLICATIONS

Biomineralization has been used in a multitude of construction applications. The most studied applications include historic preservation, soil stabilization, self-healing concrete, and production of biomineralized concrete-like alternatives. While all these applications rely on similar microbial biomineralization approaches, each process is unique, as are the challenges that must be overcome for wide-scale commercial implementation.

3.1. Biodeposition for Historic Preservation

Microbial biomineralization was first used in the building and construction industry for the express purpose of restoring historic structures, cultural heritage sites, and artworks (24, 75, 76). Over time, carbonate-based materials, like limestone and marble, experience deterioration due to weathering. Surface restoration, termed biodeposition, occurs when biomineralizing microorganisms are added or stimulated to grow on the facades of masonry structures or artworks made from gypsum or limestone. Biomineralizing microorganisms deposit $CaCO_3$ on these materials, thus creating new, durable, protective $CaCO_3$ layers. These protective layers simultaneously decrease porosity and increase mechanical strength. By decreasing water permeability, biodeposition inhibits other degrading agents from causing further damage.

As reviewed by Ortega-Villamagua and colleagues (76), biomineralization has been used to rehabilitate and preserve archaeological plasters, ancient clay roof tiles, and historic stone structures. In such applications, biomineralized surface treatments increased mechanical properties by up to 6 mm deep into treated surfaces and decreased water absorption by up to 7%. Observations over time have revealed that the surface strength has been maintained and no further deterioration has occurred years after application of the surface treatments.

In contrast to biomineralization, synthetic or chemical treatment methods for restoration and conservation applications add nonconsolidated, external coatings that require additional maintenance over time and prevent water and gas movement within stone structures, thus leading to increased internal deterioration (24, 76). Chemical coatings are often incompatible with original stone materials, and this incompatibility can cause further deterioration and introduce environmental concerns. Although biomineralization has been applied to various historic structures, it has yet to be optimized for universal restoration and conservation use.

3.2. Soil Stabilization

Biomineralization has been applied to stabilize soil in geotechnical engineering applications. Biological soil stabilization, also termed biogrouting, is currently one of the most active biomineralization research areas within the construction sector and has been the subject of multiple recent reviews (39, 77, 78). During the process of biological soil stabilization, biomineralizing microorganisms are added directly to sand or soil materials through injection, premixing, or surface percolation. These microorganisms precipitate $CaCO_3$ between soil or sand grains, thereby achieving a bioclogging effect. Some microorganisms biocement soil and sand particles with $CaCO_3$ precipitates, thus increasing soil stiffness and strength. Other benefits include decreased soil permeability and hydraulic conductivity, decreased liquefaction risk in earthquake-prone areas, improved erosion control, and stabilization of loose-aggregate pavements (34, 35, 79).

Although differences in soil type and biocementation treatment method impart significant variability in reported results, stabilized soils have achieved unconfined compressive strengths of up to 14 MPa (39). For comparison, unconfined compressive strength values for untreated soils are on the order of 100 kPa (67). Generally, higher $CaCO_3$ content increases the unconfined compressive strength of biocemented soils; however, soil stabilization may be achieved with $CaCO_3$ contents ranging from less than 1% to more than 25%, depending on factors such as soil confinement and soil particle sizes and their distribution (78).

Soil biocementation is a nascent field that faces multiple commercial challenges. The most pressing challenges relate to system design, control, and optimization, given the extreme heterogeneity across soil types and the economics of commercialization and scale-up.

3.3. Self-Healing Concrete

One of the most widely studied applications of microbial biomineralization in construction materials science is microbially induced self-healing of portland cement concrete, as evidenced by multiple recent reviews on the subject (80–83). Although portland cement concrete is widely used, it can suffer from significant durability issues. Concrete durability is negatively affected by water absorption, sulfate or chloride ion penetration, and freeze-thaw cycling. Each of these mechanisms can lead to cracking, thus necessitating extensive maintenance and repairs throughout the lifetime of a concrete structure.

The premise of self-healing concrete is that the addition of biomineralizing microorganisms to portland cement concrete, along with proper nutrients and moisture, can lead to the filling of cracks by precipitated $CaCO_3$. The biomineralizing capabilities of microorganisms have been studied as crack-healing and crack-prevention techniques. For crack healing, microorganisms are sprayed onto concrete surfaces following significant crack formation (82) in the same manner in which biomineralizing microorganisms have been applied to historic preservation. This method is limited to surface cracks and is unable to address internal microcracks formed in the bulk material. The protection of concrete material surfaces through direct application of biomineralizing microorganisms has also been explored as a preemptive crack-prevention technique because of its potential benefits in reducing the ingress of ions and strengthening and protecting the concrete surface (45, 84).

In another preemptive crack-prevention approach, biomineralizing microorganisms are directly incorporated into fresh cement paste, mortar, or concrete before it hardens. When microcracks form in the hardened matrix, biomineralizing microorganisms (encompassed in nutrient-containing capsules that rupture on cracking or that are mixed in with nutrient solutions and revived from a vegetative state by external water ingress) can seal microcracks with precipitated $CaCO_3$ before the cracks propagate into larger, more detrimental macrocracks (80, 81). To ensure crack healing, biomineralizing microorganisms must maintain viability and metabolic activity (81). Maintaining microorganism viability is the primary challenge for long-term self-healing portland cement concrete applications. Similar to the case of historic preservation applications, nutrient availability, moisture, and the physicochemical environment of microorganisms can be more easily controlled when biomineralizing microorganisms are applied to concrete surfaces. Mixing microorganisms directly into fresh portland cement paste, mortar, or concrete presents a host of challenges for living cells (80). Although many microorganisms used for biomineralization applications are moderately alkaliphilic (pH ∼ 9–10), most microorganisms used in self-healing concrete applications, such as *S. pasteurii* and other bacilli, are not adapted to the extreme chemical environment of concrete. The extreme alkalinity (pH ∼ 13) and low-oxygen environment within concrete create a particularly challenging environment for biomineralizing microorganisms. Additional challenges include microorganism access to nutrients and the elevated temperatures and pressures that occur during cement hydration. In multiple studies, only 0.01% to 0.4% of initial inocula survived after multiple days in cementitious materials due to such factors (27, 85–87). The thorough mechanical mixing needed to produce cement and concrete materials can also significantly reduce microorganism viability. Skevi et al. (88) found that, a few hours after mixing, only 0.01% of initial microorganisms added to cement mortar were still viable. This reduction in viability was likely due to the shear forces experienced by microorganisms during mixing and to the extreme environmental factors (i.e., pH shock) that cells experienced within the cement mortar. Achal and colleagues (85) showed that additive-induced aeration can improve microorganism viability. In their study, up to 16.6% of the initial inoculum survived after 3 days in concrete when 40% fly ash was added in place of cement, which the authors argue increased the number of pores and therefore aeration in the concrete specimen, leading to increased microbial viability (85). However, viability still significantly decreased over longer time frames. Even without the addition of more microorganisms, nutrients (media) and cementation resources (calcium) must continually be supplied to ensure the continuation of biomineralization within building materials. Such continuous treatment is currently feasible only at the laboratory scale.

To improve microbial viability, researchers have explored immobilization and encapsulation of microorganisms within other materials prior to adding them to portland cement paste, mortar, and concrete (89–92). Such methods involve encapsulating living microorganisms in a vegetative state or as viable spores, along with the nutrients necessary for biomineralization, prior to adding them to cement matrices. Materials used for microorganism encapsulation include biochar, rubber particles, metal and graphite nanoparticles, diatomaceous earth, expanded clay, lightweight aggregate, natural fibers, and various forms of hydrogels and microcapsules containing both microorganisms and nutrient sources (51, 81, 90).

The effect of encapsulation on microbial viability within the cementitious matrix is ultimately dependent on the type of encapsulation material, the encapsulation and concrete casting methods, and whether additional nutrients were applied throughout the curing process. Concrete specimens incorporating encapsulated biomineralizing microorganisms have been reported to fully heal cracks ranging from 0.4 to 1.2 mm over 28 days (81, 93, 94). Shaheen et al. (94) reported full closure of a 1.2-mm concrete surface crack by using Fe_2O_3 nano/microparticle encapsulation of ureolytic biomineralizing microorganisms. The concrete also recovered 85% of its original precrack compressive strength post-biomineralization treatment. This degree of crack healing, however, was possible only through full immersion curing. While such curing is common for testing microbial self-healing in concrete materials, it is not feasible for large-scale industrial self-healing applications. While Shaheen et al. found that encapsulation improved viability and self-healing, some encapsulation methods lead to reduced microbial performance relative to direct, unencapsulated microbial addition. For example, Xu & Wang (93) found that encapsulating ureolytic bacteria in a mixture of calcium sulfoaluminate cement and silica fume led to reduced microbial activity relative to unencapsulated spores, as measured by urea decomposition during setting. Encapsulated nutrients, such as yeast extract, may also act as a set retarder and may hinder portland cement hydration (95). Additionally, encapsulation materials, such as natural fibers, may act as nucleation sites for hydration products, thus affecting the final hardened-state properties. Calcium sources required for biomineralization can also affect cement hydration and durability; calcium acts as a hydration accelerator, and chloride (added when $CaCl_2$ is added as the calcium source) can be detrimental to concrete durability.

Despite improvements in encapsulation approaches, microorganism viability within self-healing portland cement concrete exponentially decreases over time (96), limiting the self-healing life span of such materials, whether or not the microorganisms are encapsulated in other materials. This result casts doubt on the actual commercial feasibility and long-term performance of self-healing concrete utilizing currently available microorganisms and incorporation approaches. Fundamental research is needed to investigate the mechanisms and effectiveness of nonviable microorganisms or microbial enzymes for self-healing applications, which may prove more successful and applicable at the commercial scale.

3.4. Other Applications

In addition to surface applications, soil stabilization, and self-healing portland cement concrete, biomineralizing microorganisms are being exploited as a cementing and strength-improvement mechanism in a variety of other building materials, such as recycled aggregates, rammed earth, and compressed earth blocks. Recycled aggregates, typically generated by crushing waste concrete, suffer from significant water absorption and bonding challenges due to the adherence of hardened, porous cement paste on the aggregate surface (97). When incorporated into new concrete, highly porous recycled aggregates can affect concrete workability and compressive strength. Researchers have explored surface treatments to reduce water absorption and increase the interface compatibility of recycled aggregates (97, 98). While traditional approaches include mechanical or chemical treatments, recent research has explored the use of biomineralization as a more sustainable treatment to improve the surface properties of recycled concrete aggregate. Initial studies have shown that biomineralization treatments can increase recycled aggregate strength and decrease water absorption, producing concrete with properties similar to those of concrete made with virgin aggregate (97, 98).

Researchers have also proposed the use of biominerals as a binding material for rammed earth and compressed earth blocks (99). Rammed earth construction relies on the compaction of earth that is often mixed with a cementing material. Recent research has explored the use of biomineralizing microorganisms in biocement rammed earth construction materials (53, 100). Fang & Achal (53) reported an ∼42% increase in compressive strength and an ∼27% decrease in water absorption for cement-stabilized rammed earth blocks treated through biostimulation of biomineralizing ureolytic microorganisms. Interlocking compressed earth blocks can also be stabilized through biomineral precipitation (101). While these studies show promise in creating stronger and more durable rammed earth and compressed earth block building materials, a fundamental understanding of the structure-property relationships of biomineralized load-bearing earth materials and structures is still in its infancy. Building materials made from such biomineralized, low-energy binders show promise as a replacement for traditional, high-energy building materials, such as clay bricks, with compressive strength on the order of 5 MPa. However, these building materials have not yet achieved the mechanical or durability performance required to replace conventional portland cement concrete (which has compressive strength on the order of 50 MPa) due to the enhanced strength of cement hydration products and their interaction with aggregates.

3.5. Process Innovations for Biomineralized Building Materials

While bioaugmentation and biostimulation have been well studied and well developed at the laboratory scale, a newly emerging, and perhaps transformative, extension of bioaugmentation of biomineralized brick fabrication is 3D printing. Nething et al. (102) designed and fabricated one of the first spatially patterned biocemented structures by using 3D printing. These researchers printed a biomineralizing sand mixture containing lyophilized biomineralizing ureolytic microorganisms. After printing, the printed structure was exposed to a solution containing calcium chloride and urea. Printed areas containing the biomineralizing sand ureolytically produced $CaCO_3$, which cemented the sand particles together to form a solid structure. Printed sand that did not contain the biomineralizing powder did not solidify. Nething et al. acknowledged that the optimization of 3D printing parameters (e.g., layer height, print speed) and increased sand density (i.e., particle size distribution) could improve the 3D printing process and increase the quality and strength of the final 3D printed structure.

In addition to investigating 3D printing, researchers are now paying more attention to the continuous supply of nutrients in biomineralized building materials. While the single-dose nutrient approach is preferred, multiple-dose treatment options offer the benefit of increased microbial viability and biomineralization efficacy. Multiple treatments refer either to the addition of nutrient solutions to feed the initial microbial inoculum or to the addition of nutrient solutions along with additional microorganisms (56, 69, 103). Understanding microbial viability and the effects of nutrient addition is paramount to fully understanding biomineralization efficacy. However, most studies apply microorganisms and nutrients only once. Some researchers have rigorously studied the effect of different treatments on the viability of microorganisms over time (64, 82, 88). A fundamental understanding of how long specific microorganisms retain metabolic activity within specific material systems, without or with additional doses of nutrients or microorganisms, can help materials engineers fully leverage microbial biomineralization to produce materials with consistent and predictable mechanical properties and long-term durability.

3.6. Compressive Strength and Water Absorption of Biomineralized Building Materials

The most important property measurements for load-bearing building materials are compressive strength, which is a measure of load-carrying capacity, and water absorption, a common proxy for durability. Compressive strength and water absorption values for biomineralized building materials are depicted in **Figure 4**, using data from a meta-analysis of 82 studies (**Supplemental Table 1**) that reported the effect of biomineralizing organisms on the compressive strength and water absorption of biological mortars, concretes, and other load-bearing materials (e.g., compressed earth blocks, stabilized sand) (40, 44, 46, 47, 50–53, 57, 59–68, 70–72, 85, 89–91, 93, 95, 101, 104–156). Data for the biomineralized materials are plotted as a percent increase or decrease relative to their respective nonbiological controls.

Supplemental Material >

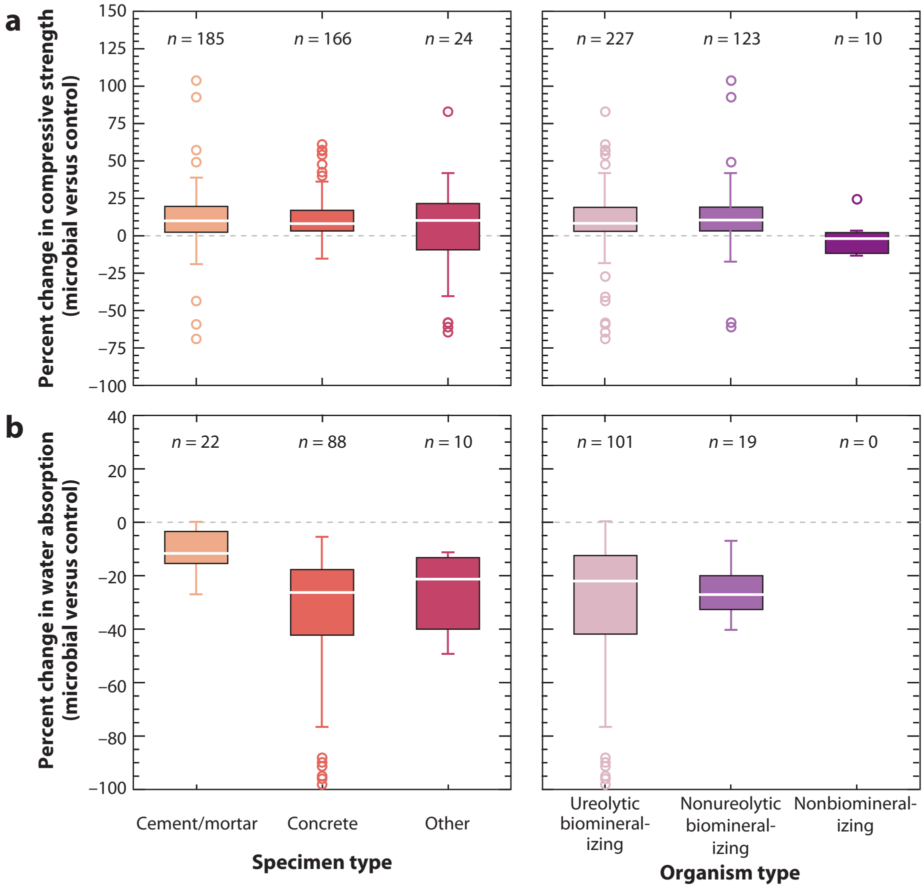

Figure 4

Differences in (*a*) compressive strength and (*b*) water absorption between microbial biomineralized building materials and their respective nonbiological controls. Data were collected from References 40, 44, 46, 47, 50–53, 57, 59–68, 70–72, 85, 89–91, 93, 95, 101, and 104–156.

In general, the addition of biomineralizing microorganisms to these building materials increased the 28-day compressive strength—the standard measurement for cementitious materials—and decreased water absorption relative to nonbiological controls. However, significant variability between studies exists. This variability can be attributed to the large range of additional, confounding variables present in all biomineralized building material studies, including the type of organism, the nutrient solutions, the treatment methods, the number of treatment cycles, and the mixture design of the material.

For all types of biomineralized building materials reported in **Figure 4*a***, the median reported compressive strengths increased marginally relative to the respective control samples. Median increases are observed for all sample types, with the largest median increases shown for portland cement mortar samples and other types of biobricks (~10% each), followed closely by portland cement concrete, with a median compressive strength increase of ~8%. Almost all portland cement mortar and concrete studies analyzed in **Figure 4** reported increases in compressive strengths relative to the control materials.

As expected, the addition of biomineralizing microorganisms consistently decreased water absorption in all sample types. The largest median decrease in water absorption was observed for concrete samples (~26%), followed by other types of biocemented sands and soils (~21%). Cement mortar specimens showed a slightly lower median decrease in water absorption (~11.6%). Almost all studies that reported water absorption tests used ureolytic microorganisms. Biomineralized building materials using ureolytic organisms exhibited a median water absorption decrease of ~22%, whereas studies using nonureolytic organisms exhibited a median water absorption decrease of ~27%.

Sixty-two of the 82 studies reviewed selected a ureolytic microorganism, while 27 of the 82 studies used nonureolytic microorganisms (**Supplemental Table 1**). As shown in **Figure 4*a***, ureolytic organisms within biomineralized building materials increased the median compressive strength of nonbiological controls by ~8% versus ~10.5% for specimens incorporating nonureolytic biomineralizing microorganisms The largest reported increase in compressive strength for ureolytic specimens was >57% (46, 151). For nonureolytic cement specimens, the increase exceeded 103% (131). While some studies reported significant decreases in the compressive strength of specimens incorporating microorganisms relative to their nonbiological controls, the majority of biomineralized building material studies reported increases (67, 114, 119).

Supplemental Material >

3.7. Other Microorganism Effects

Some well designed studies report the effects of nonbiomineralizing microorganisms, most commonly *Escherichia coli*, as a negative biological control (**Figure 4*a***) (52, 105, 147). The median compressive strength change for specimens incorporating nonbiomineralizing microorganisms was approximately −2%. Despite this median decrease, a large range of values are reported for nonbiomineralizing microorganisms (−13% to +24%). These results are highly dependent on the microorganism selected. *E. coli* is generally accepted to cause no increases or decreases in compressive strength in biomineralized samples. However, Park et al. (111) report compressive strength increases of >24% for bioblocks treated with biofilm-forming, nonbiomineralizing microorganisms. In that study, the biofilm-forming, nonbiomineralizing microorganisms showed strength increases even greater than those shown for ureolytic biomineralizing *S. pasteurii*. Park et al. conclude that mature biofilms may act as binder materials to strengthen cementitious materials without the need for biomineral precipitation. More research is needed to elucidate the strengthening mechanisms, the effects of different biofilms, long-term durability, and microbial viability in such systems.

Because building materials containing biomineralizing microorganisms often possess higher compressive strength than do control samples, most researchers have assumed that such strength increases are directly caused by precipitated minerals within the material matrix. However, Skevi et al. (88) recently called this assumption into question. Alongside reports of very low microorganism viability after a few hours of mixing (e.g., 0.01% after 3 h) within a cement mortar mixture, the authors report 28-day compressive strength increases of up to 19% over control mortar specimens when dead cells were incorporated instead of living cells. Although specimens containing live cells showed higher increases in compressive strength (32%) over control specimens than did those containing dead cells, the authors concluded that strength increases appear to be largely independent of cell viability.

Skevi et al. (88) propose that the presence of cell matter within the cementitious matrix can act as nucleation sites for cement hydration product formation, thus increasing compressive strengths of mortars containing either live or dead cells. Cell materials, such as proteins and metabolic byproducts, may also be reacting directly with ions present in the cement pore solution, in which the highly alkaline pH drives mineral precipitation independently of microbial metabolism. These conclusions are supported by earlier work conducted by Williams and colleagues (27), in which the authors quantify and report significant calcite formation in both cement pastes originally prepared with live cells (∼16%) and those incorporating dead cells (∼12%). Skevi et al. (88) also highlight the effects of complex interactions between microorganism strain, nutrient solution, and fabrication method.

Taken together, these results, as well as the contradictory results of decreased strength with living biomineralizing cells evidenced in other studies (**Figure 4**), highlight the need for additional research designed to specifically elucidate the mechanism(s) of action related to the effects of nonliving, nonbiomineralizing cells and their media. Do microorganisms have to be alive to precipitate biominerals? Are the increases in initial compressive strength and self-healing observed and reported in the literature attributable to the metabolic activity of living organisms?

4. EMERGING IDEAS IN BIOMINERALIZATION OF BUILDING MATERIALS

While significant research attention is still focused on traditional approaches to biomineralization and its applications in construction, new biomineralization approaches and applications have arisen in recent years.

4.1. Living Building Materials

In an effort to address the drawbacks of reduced viability in biological portland cement mortar and concrete materials, Heveran et al. (41) introduced the idea of continuous living building materials made from sand, gelatin, and biomineralizing photosynthetic organisms. Such continuous living building materials (LBMs) were designed to maintain microorganism viability over extended periods of time (157). The LBMs engineered by Heveran et al. maintained 9–14% microbial viability over 30 days when kept at 50% relative humidity. The authors also demonstrated that exponential manufacture is feasible if biological building materials can be kept alive. Specifically, after inoculating one sand-gelatin scaffold and producing one parent LBM generation, the researchers produced three additional LBM generations (eight total LBMs) without the addition of microorganisms. In other words, one parent generation produced two LBMs. These two LBMs then were split into halves to produce four total LBMs, and these four replicated to produce a total of eight child generation LBMs from one parent inoculum.

A follow-up study by Qiu et al. (40) reported properties of LBMs that were made using both photosynthetic and ureolytic microorganisms. In this study, a desiccation protectant was added. This resulted in a 3% microbial viability rate of the photosynthetic organisms after 30 days at ambient conditions. This research also reported an improvement in mechanical properties, such as compressive strength and fracture energy, by tailoring the sand-gelatin matrix ratios. Although the ∼2–5-MPa compressive strength of these LBMs is still too low to replace structural concrete, the addition of coarse aggregate would likely make these materials more directly competitive. Nevertheless, the studies by Heveran et al. (41) and Qiu et al. (40) represent a critical departure for the field of construction biotechnology and an emerging area of research that shows significant promise.

4.2. Martian Biomineralized Concrete

Researchers are exploring emerging biomineralization application areas related to space exploration and colonization of other planets. Lower resource burden, namely not having to directly transport portland cement or concrete materials, is the primary advantage of using microorganisms to produce building materials for extraterrestrial applications. In an initial study, Gleaton et al. (158) analyzed the biogrouting ability of *Thraustochytrium striatum*, a microorganism that can both hydrolyze urea and use acetate as a carbon source, in the context of simulated martian regolith. This study explored the potential of anaerobically digesting martian rocks to produce calcium acetate for such biomineralization applications. The results showed that *T. striatum* was able to use this calcium acetate as the sole carbon/calcium source for biomineralization; biogrouted sand columns showed an up-to-95% reduction in hydraulic conductivity with such an approach. These results indicate that nonureolytic microorganisms can utilize inexpensive, space-generated nutrient sources and can effectively biocement simulated martian regolith specimens. Given that this research was the first study of its kind, additional studies are needed to optimize biocementation of simulated martian regolith and to explore specific building applications of such a technology in the aerospace industry. Although such applications are interesting and will likely be necessary for future space exploration programs, biomineralization applications on Earth must be elucidated and well understood prior to extraterrestrial applications.

4.3. Synthetic Biology

Researchers are exploring the application of synthetic biology to enhance—or introduce—microorganisms' biomineralization capabilities to produce biomineralized building materials. Studies by Sarkar et al. (117) have examined genetically modifying nonbiomineralizing *E. coli* with biosilification proteins, leading to strength increases in mortar samples of up to ∼31%. The aforementioned LBM work by Qiu et al. (40) utilized a bioengineered strain of *E. coli* as a ureolytic biomineralizing microorganism. Genetic engineering not only can introduce biomineralization capabilities to nonbiomineralizing microorganisms but also can result in greater control over the $CaCO_3$ biomineralization process. For example, Heveran and colleagues (37) demonstrated that certain strains of engineered *E. coli* can precipitate different $CaCO_3$ polymorphs with distinct nanomechanical properties.

4.4. Other Biological Approaches

Emerging avenues of biocementation research directly target the avoidance or reduction of CO_2 emissions attributable to portland cement production. In one study, Røyne et al. (159) produced a $CaCO_3$ biogrout through two microbial steps. The first step utilized soil-isolated, organic acid–producing (pH 6.0–9.5) bacteria closely related to *Bacillus safensis* to dissolve powdered limestone ($CaCO_3$) into Ca^{2+} and $CO_3{}^{2-}$ ions. The second step utilized the ureolytic metabolism of *S. pasteurii* to reprecipitate Ca^{2+} and $CO_3{}^{2-}$ as a $CaCO_3$ biogrout to cement sand into a concrete-like alternative. This method produces a $CaCO_3$ binder that avoids the calcination-related CO_2 emissions associated with portland cement production. Early prototypes, termed BioZEment, show 0.2–1.2-MPa compressive strength after 40 microbial and nutrient solution injection treatments (159). While the environmental benefits may prove promising, low compressive strengths and the multiple required treatment cycles are disadvantages of this approach. In addition, the final pH of biocemented mortars is a significant challenge. Pure $CaCO_3$ binders exhibit increased brittleness and lower internal pH relative to portland cement concrete, which can lead to corrosion issues with embedded steel reinforcement.

5. CHALLENGES AND FUTURE DIRECTIONS

Despite proofs of concept at the laboratory scale, the field of biomineralized materials for construction faces a multitude of challenges (20, 21, 160). While challenges at the laboratory scale include material optimization (i.e., microorganism growth and biomineral production) and property enhancement, most challenges exist at the commercial scale. The most significant challenge concerns the safe and sustainable application of microorganisms outside of a controlled laboratory setting (161). Intentional application of non-native microorganisms through bioaugmentation in large-scale construction applications, such as soil stabilization and self-healing concrete, raises significant environmental concerns. Applications of natural microorganisms or genetically modified microorganisms introduce significant biodiversity, human health, and biosafety concerns. Microorganisms originating from native soil environments may pose less concern than genetically modified microorganisms for such applications; however, such organisms are not native to all soil environments, and their release may still significantly alter native ecosystems. The release of microorganisms from a laboratory, even the intentional application of nonpathogenic soil microorganisms, is likely to raise public speculation. Additionally, metabolic by-products of biomineralizing microorganisms, such as ammonia and hydrogen sulfide, are considered environmental contaminants and would have to be avoided or closely monitored and controlled in construction biotechnology applications.

From a regulation standpoint, the use of biomineralizing microorganisms in construction biotechnology must follow biosafety laws and regulations, including proper transportation and handling of microorganisms, and may require the performance of risk assessments for each application. Environmental rules and regulations also vary significantly between countries, as well as on a more local level, adding additional nuance to applying such technologies on an industrial scale. Most importantly, to enable the rapid adoption of new materials, biomineralized building materials must also meet construction codes and specifications that vary between countries and that are slow to change.

Apart from safety and ecosystem health concerns, scale-up feasibility and cost realism are two of the most prominent challenges facing the industrial implementation of biomineralized building materials. Most microorganisms used in construction biotechnology rely on potable water for growth and biomineralization; the use of potable water can be attributed to a lack of exploration and applications of marine microorganisms relative to terrestrial microorganisms (162). While biomineralizing marine microorganisms and their saltwater dependence have yet to be applied more broadly in the biomineralized building material field, it is hypothesized that biomineralization using marine microorganisms may become an important area of research in the coming years.

The nutrients required for microorganism growth are the single largest contributor to the cost of biomineralized building materials. Because ureolytic microorganisms are heterotrophic and require additional cementation resources (e.g., urea), they require more material and cost inputs than do microorganisms that utilize other biomineralization pathways (e.g., photosynthesis). Despite these costs, a host of recent studies have detailed the successful use of waste materials (e.g., lactose mother liquor, corn steep liquor, tofu wastewater, urine) as nutrient sources for biomineralizing microorganisms (63, 64, 103, 149, 163–165). The continued shift to waste materials will help keep costs lower and potentially provide an additional treatment option for waste materials as these technologies scale. The reliance on waste streams from other industries may eventually prove to be a hindrance to the scale-up and implementation of construction biotechnology if there are changes to material processing or supply chain disruptions. Such challenges have begun to occur in the cement and concrete industry with the phasing out of coal-fired power plants, which have supplied fly ash as a supplementary cementitious material to the industry for the past several decades.

While photosynthetic microorganisms require fewer nutrients than do heterotrophic microorganisms, the light and land requirements necessary for their growth introduce unique challenges.

Photosynthetic microorganisms can be cultured at scale industrially using natural sunlight, but geographic location can introduce significant variability. Furthermore, because photosynthetic microorganisms require access to light, large swaths of land will be required to grow them at scale. However, unlike other bio-based construction materials, such as bamboo, hemp, and wood, photosynthetic microorganisms can be cultivated on nonarable land and typically show greater biomass productivity than do terrestrial crops (166, 167). The industrial-scale cultivation of microorganisms solely for biomineralization applications may not be economically feasible. However, biomineralizing microorganisms also produce organic biomass throughout their life cycles, and such biomass can be cultivated as valuable coproducts, such as biofuel or food resources, alongside produced biominerals, thus increasing economic feasibility (168). Production of coproducts may prove a significant challenge but is likely necessary if photosynthetic biomineralization is to become a commercial-scale construction technique.

While biomineralization is generally considered an environmentally low-impact approach that will increase the sustainability of the construction industry and ultimately reduce associated CO_2 emissions, this claim has yet to be substantiated. Detailed product-based life cycle assessments are vital to confirm such claims before biomineralized building materials are accepted as a greener alternative to traditional construction materials.

The primary challenge facing the field of construction biotechnology is that it currently relies on the cooperation of multiple disciplines that largely work independently. Biology, civil engineering, and materials science have historically worked within their own fields (169). In many instances, biomineralized materials research involves researchers from one discipline (such as biology) attempting to understand and apply their discipline to applications in a separate discipline (such as civil engineering). While research teams consisting of individuals from disparate disciplines have been able to develop and work toward interdisciplinary research goals within the academy, a disconnect still exists between interdisciplinary research at the academic level and the feasibility of commercial application. This paradigm must shift if biomineralized materials are to become a new normal for the construction industry. To create such a shift, individuals from biology, industrial biotechnology, civil engineering, industrial engineering, and materials science must join together to create the new discipline of construction biotechnology (170). As a first step in this direction, Pacheco-Torgal & Labrincha (171) propose significant updates to the standard university civil engineering curriculum, including the incorporation of biotechnology and nanotechnology principles.

Despite the significant challenges that may lie ahead for the field of construction biotechnology, substantial progress has made since seminal studies were first published in the 1990s. The number of researchers, funding agencies, and startup companies [e.g., Ecovative (**https://ecovative.com**), Basilisk (**https://www.basiliskconcrete.com/en/**), Biomason (**https://biomason.com**), Minus Materials (**https://www.minusmaterials.com/**), Prometheus Materials (**https://prometheusmaterials.com**)] dedicated to this field has substantially increased over the past decade, in large part due to a steadfast belief that biotechnology will play a significant role in the fight against climate change, particularly for the construction industry.

DISCLOSURE STATEMENT

D.N.B and S.L.W. do not have any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review. W.V.S. is a listed coinventor on a patent application (PCT/US2020/020863) filed by the University of Colorado on April 3, 2020, related to biomineralized building materials. W.V.S. is a cofounder and shareholder of Prometheus Materials and Minus Materials and a member of their scientific advisory boards.

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